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A Biochemical, Histologic, and Immunohistologic Analysis of Membranes Obtained From Failed Cemented and Cementless Total Knee Arthroplasty

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Biochemical, histologic, and immunohistochemical analyses were performed on 34 interface membranes obtained from 33 patients during revision total knee arthroplasty. The membranes had surrounded components of cementless (n = 11) and cemented (n = 23) knee prostheses that were aseptically loose. None of these implant failures was caused by catastrophic polyethylene erosion leading to metal-to-metal contact. The histologic findings were similar in the membranes from cemented and cementless knee components: small polyethylene debris within macrophages and large birefringent polyethylene debris within foreign-body giant cells. Metallic debris was seen in membranes from both groups, but cemented membranes had more polymethylmethacrylate particles and more hyalinization. Intracytoplasmic asteroid bodies were observed in several foreign-body giant cells in both types of membranes. No significant differences were found between the two groups in levels of collagenase, prostaglandin E_2 (PGE_2), interleukin-1 (IL-1), interleukin-6 (IL-6), or tumor necrosis factor-alpha (TNF-α), nor in the population of inflammatory cells stained with IL-1, IL-6, and TNF-α antibodies. Membranes that had surrounded components with radiographic evidence of diffuse or localized periprosthetic bone loss released significantly more collagenase, IL-1, IL-6, and TNF than did membranes from components without bone loss. These two groups, however, did not have significantly different PGE_2 levels. These findings suggest that polyethylene and metal debris may play a role in macrophage activation and the release of mediators of bone resorption in the membranes surrounding failed cemented and cementless total knee implants.

Aseptic loosening and osteolysis have increased as the premier obstacle limiting the longevity of total joint replacements. Initially thought to be inherent to the use of polymethylmethacrylate (PMMA) cement, osteolysis is now recognized in both cemented and uncemented arthroplasties. Its occurrence is well documented in total hip arthroplasty, and is believed to be the result of a foreign body reaction to particulate PMMA, metal, and polyethylene wear debris.[7,11,13,15,17–20,23,28] There is also evidence that several biochemical factors associated with bone resorption such as collagenase, prostaglandin E_2 (PGE_2), interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF), may play an important role in the development of aseptic loosening and osteolysis.[5,7,15,17]

The problem of osteolysis has not been as well described in total knee arthroplasty (TKA). Recently, however, investigators have noted osteolysis around loosened cementless total knee prostheses.[14,21] These re-

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ports have described the presence of bone–implant interface membranes with similar histology to those reported in cases of osteolysis around cementless total hip prostheses. Biochemical analyses of these membranes from total knee replacement have not been documented, however. The current study was conducted to determine the biochemical, histologic, and immunohistochemical characteristics of interface membranes surrounding failed cementless and cemented TKAs (referred to as "cementless membranes" and "cemented membranes", respectively).

MATERIALS AND METHODS

Thirty-four interface membranes were harvested from 33 patients undergoing surgery to revise failed total knee prostheses at the University of Pittsburgh Medical Center and the Pennsylvania Hospital between July 1991 and April 1992. Twenty-three membranes (22 patients) were taken from cemented implants: five porous-coated anatomic, ten Insall-Burstein, three Miller-Galante, and five Johnson & Johnson prostheses. This group comprised 12 men and 11 women whose mean age was 65.7 years (range, 47–81 years). The 11 cementless membranes were taken from nine Miller-Galante and two LCS Rotating Platform implants. The seven women and four men in this group had a mean age of 63.5 years (range, 38–83 years). To serve as a control group for the biochemical analysis, ten membranes (four cementless and six cemented) were harvested from the fibrous pseudocapsule of ten additional patients undergoing revision knee arthroplasty. All components in all three groups articulated with a polyethylene tibial and patellar surface. There were no patients with carbon-impregnated polyethylene.

The average time between the initial arthroplasty and revision procedure was 60.1 months (range, 49–109 months) in the cemented group and 80.6 months (range, 26–96 months) in the cementless group. The primary indications for the first arthroplasty were osteoarthritis (13 cemented and eight cementless) and rheumatoid arthritis (ten cemented and three cementless), whereas the indications to perform all revision procedures were knee pain and radiographic findings of component loosening or migration. The latter was defined, on preoperative radiographs, in accordance with the Knee Society system as the presence of a radiolucent line at least 1 mm wide at the bone–cement or bone–implant interface (Fig. 1).[6,26] The widths of radiolucent lines were measured on the basis of seven zones on an anteroposterior view of the tibia; three on a lateral view of the tibia and seven on a lateral view of the femur. The widths of radiolucent lines on a tangential radiograph of the patella were not measured. Among the 23 cemented implants, eight femoral components and 13 tibial components met these criteria; in the 11 cementless implants, the numbers were three femoral and six tibial components (Table 1). Intraoperative examination confirmed that all of these components were loose and surrounded by a fibrous membrane. Loosening was confirmed intraoperatively by using manual pressure to elicit visible motion at the bone–implant interface.

All patients in this study had discontinued taking antiinflammatory medications ten days before revision surgery. In addition, preoperative and intraoperative cultures of aspirates from the hip joint were sterile for all patients.

All membranes were cut into portions for separate biochemical, histologic, and immunohistochemical analysis.

Histology

Periprosthetic membrane samples were prepared for hematoxylin and eosin (H&E) staining. Paraffin-embedded specimens were analyzed qualitatively under regular light and polarized microscopy for macrophages, fibroblasts, particulate PMMA, metal debris, and polyethylene debris. Using a modification of Mirra's grading system,[15,20] each sample was graded according to the number of cells and particles present (0 = absent, 1+ = mild, 2+ = moderate, 3+ = severe).

Biochemical Assays

The membrane specimens were cut into small fragments, and organ cultures were performed using a modified Goldring method.[6] After three days of incubation, the culture media were recovered and assayed for collagenase, PGE_2, IL-1, IL-6, and TNF-α.

Collagenase activity was measured using post-synthetically labeled [^{3}H] acetic anhydride (New England Nuclear, Boston, Massachusetts) as a substrate. The detailed technique was reported previously.[15,27] One unit of collagenase activity degrades 1 μg per minute of substrate. Prostaglandin E_2 was measured by radioimmunoassay with a commercially available kit (New England Nuclear), and IL-1 was measured by bioassay using the proliferation of the D10.G4.1 T helper clone, as described elsewhere.[15,25] Finally, IL-6 and TNF-α were measured with a commercially available enzyme-linked immunosorbent assay (ELISA) kit.

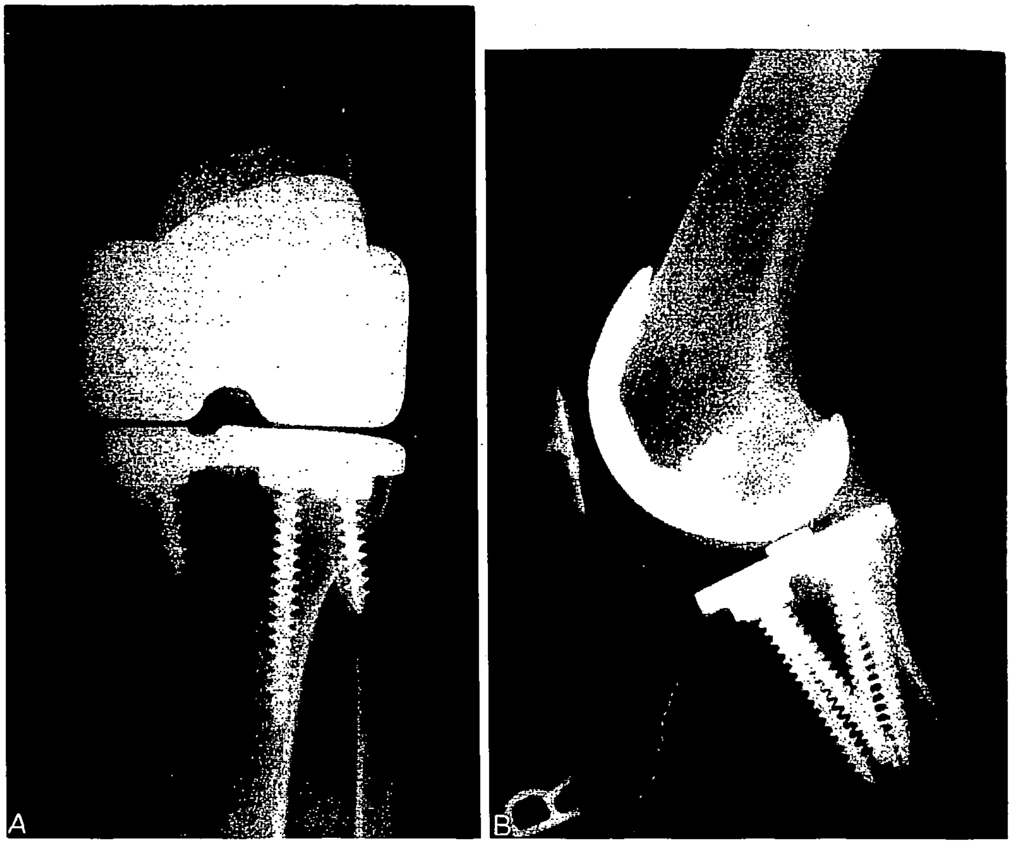

FIG. 1. Anteroposterior and lateral radiograph of a loosened cementless TKA.

The data thus obtained were statistically compared with a Student's t-test.

IMMUNOHISTOCHEMISTRY

To clarify which cells were producing IL-1, IL-6, and TNF-α in the interface membranes, immunohistochemical analysis was performed using anti–IL-1, anti–IL-6, and anti–TNF-α antibodies. The specimens in this study were frozen in OCT compound (Lab-Tek Products, Naperville, Illinois) and stored at −70°. Sections of the frozen tissue were cut 7 μm thick on a cryostat, fixed in cold acetone (4°) for ten minutes, and then dried. After these sections were washed in phosphate-buffered saline (PBS) for five minutes, they were incubated for ten minutes in an avidin- and biotin-blocking reagent (Vector Laboratory, Burlingame, California). They were washed again in PBS for five minutes and incubated with normal horse serum for 30 minutes at room temperature with monoclonal antibodies to macrophages, B lymphocytes (anti-CD3), and T lymphocytes (anti-CD22) (DAKOPATTS, Glostrup, Denmark) and polyclonal antibodies to IL-1, IL-6, and TNF-α (Calbiochem, La Jolla, California). Endogenous peroxidase was neutralized with 0.05% hydrogen peroxide for ten minutes. Next the biotinylated secondary antibody (Vector Laboratory) was applied to the sections for 45 minutes at room temperature. Then they were washed with PBS, after which 3,3′-diaminobenzidine substrate (Sigma, St. Louis, Missouri) with 0.05% hydrogen peroxide was added for color development. Finally, the sections were rinsed for five minutes, exposed to hematoxylin, and mounted with Immunomount (Shandon, Sewickley, Pennsylvania). Control specimens had the same preparation except that the first antibodies were omitted.

TABLE 1. Percentage of Knees With Radiolucent Lines According to Zone

	Radiographs					
	Lateral of Femur		*Anteroposterior of Tibia*		*Lateral of Tibia*	
Zone	*Cemented*	*Cementless*	*Cemented*	*Cementless*	*Cemented*	*Cementless*
1	5	3	6	3	2	1
2	3	0	4	3	1	1
3	0	0	2	0	0	0
4	0	0	1	0		
5	0	0	0	0		
6	0	0	0	0		
7	0	0	0	0		

RESULTS

Radiographic and Intraoperative Findings

Eight of the 23 cemented femoral components and 13 of the 23 cemented femoral components had a radiolucent line of at least 1 mm at the bone–cement interface. Similarly, three of the 11 cementless femoral components and six of the 11 cementless tibial components were loose by radiographic criteria (Table 1). Intraoperative examination confirmed that these components were loose and surrounded by a fibrous membrane.

Histologic Findings

In both the cemented and cementless membranes, microscopic examination showed macrophage infiltrates, multinucleated foreign-body giant cells, and large accumulation of debris (metal, polyethylene, or cement) within dense, organized connective tissue stroma. Metal debris was seen in membranes from both experimental groups, and most of it was intracellular within macrophages. In addition, the particulate metal frequently was confined to the membrane surface adjacent to the implant (Fig. 2). Particulate polyethylene was universally present in membranes from both groups. Many small polyethylene particles were contained within the cytoplasm of macrophages; larger particles were engulfed by foreign-body giant cells. Membranes from cemented prostheses generally contained PMMA particles and increased tissue hyalinization. Furthermore, most of the polyethylene particles were lo-

FIG. 2. Light microscopy of the specimen taken from Zones 1 and 2 around the tibial component demonstrates particulate metal debris, which is limited adjacent to the implant surfaces.

cated within the specimen where it faced the bone. Interestingly, the large particles of birefringent polyethylene appeared to occupy the periphery of the membrane. Several of the foreign-body giant cells in both types of membranes also contained intracytoplasmic asteroid bodies (Fig. 3). The control tissues were composed of a dense collagenous stroma containing no wear particles and few inflammatory cells.

BIOCHEMICAL FINDINGS

The collagenase activity in culture media recovered from the cementless membranes ranged from 1.8 to 14.4 units per gram of tissue with a mean value (± standard error of the mean [SEM]) of 5.93 ± 5.17. For the cemented membranes, the range of collagenase activity was 1.2–13.7 units per gram of tissue, with a mean of 5.79 ± 4.47. The values for both experimental groups were not significantly different. Both sets of values were significantly higher ($p < 0.01$) than those for control tissues, however, which ranged from 0 to 7.3 units per gram of tissue, with a mean of 2.3 ± 1.2 (Fig. 4A).

Likewise, the amounts of PGE_2 in cemented membranes (range, 0–13.9 ng/mg of

FIG. 3. Asteroid bodies (short arrows) and large pieces of birefringent polyethylene debris (long arrows) are visible within foreign-body giant cells. At least two asteroid bodies can be seen in this plane of focus. (Stain, hematoxylin and eosin; original magnification, ×400.)

tissue; mean, 5.52 ± 3.75) and cementless membranes (range, 1.4–14.3 ng/mg of tissue; mean, 4.94 ± 3.83) were not significantly different. Again, both sets of values were significantly higher ($p < 0.05$) than control tissue values (range, 0–3.01 ng/mg tissue; mean, 1.93 ± 1.01) (Fig. 4A).

The activity of IL-1 was measurable in six of the 11 cementless membranes (range, 0–3.9 units per 100 mg of tissue; mean, 0.52 ± 0.57) and in 11 of the 23 cemented membranes (range, 0–0.66 units per 100 mg tissue; mean, 0.39 ± 0.82). There was no significant difference between these two groups, but both produced significantly higher IL-1 activity than did control tissues ($p < 0.05$), in which activities were uniformly undetectable (Fig. 4B).

Interleukin-6 activity was detected in all 11 cementless membranes (range, 0–303.5 ng/g tissue; mean, 123.1 ± 108.4) and all 23 cemented membranes (range, 0–311.7 ng/g of tissue; mean, 102.6 ± 103.1). Tumor necrosis factor-alpha activity was detected in eight cementless membranes (range, 0–5.9 ng/g tissue; mean, 1.63 ± 1.48) and 19 cemented membranes (range, 0–9.4 ng/g tissue; mean, 2.03 ± 3.29). There was also no significant difference between these two groups in either factor, and again, both groups had higher levels of both factors as compared with control specimens (Fig. 4B and 4C).

Table 2 compares the activity levels of the biochemical factors as measured in culture media recovered from the membranes. Levels of collagenase, PGE_2, IL-1, IL-6, and TNF-α did not differ significantly between experimental groups, but both of these groups had significantly higher levels of all of these than did the control group.

Both linear and lytic bone loss were found in association with failed femoral and tibial implants, regardless of whether the prosthesis was cemented. Postoperative radiographs showed that the medial aspect of the tibial metaphysis (Zones 1 and 2) was the most common site for osteolytic bone resorption. Intraoperatively, all of these implants were

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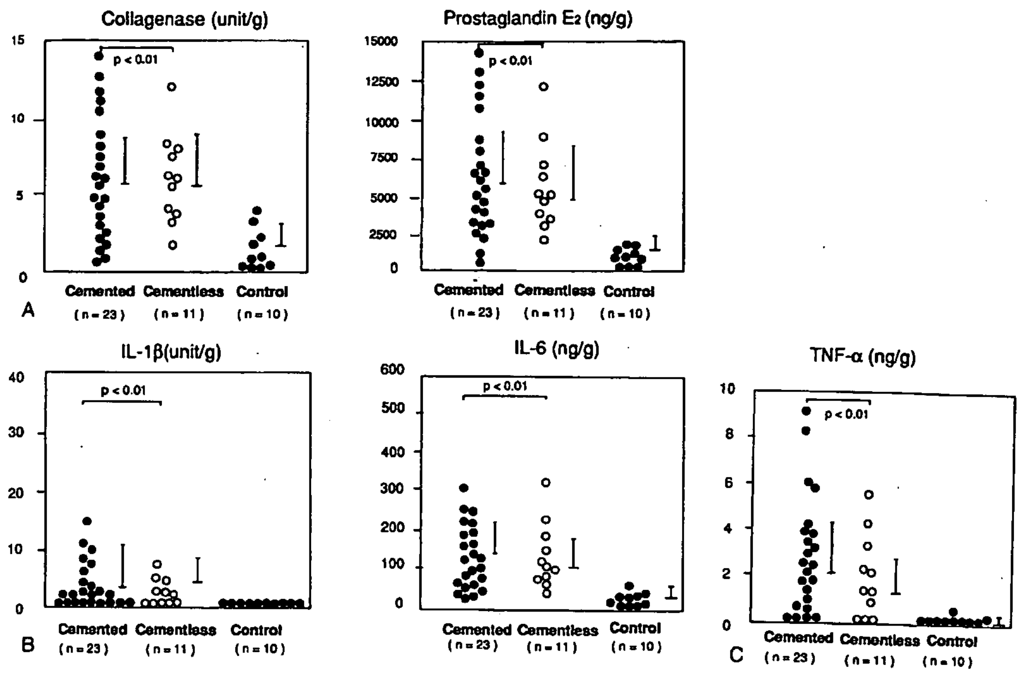

FIGS. 4A–4C. The results of biochemical comparison among cemented membranes, cementless membranes, and control tissues, represented as amounts produced per gram of membrane. Closed circles, cemented membranes; open circles, cementless membranes. The mean value and standard deviation (SD) in each group are indicated, respectively, by a vertical bar with a long and short horizontal bar beside the circles.

found to be loose. Four cases of focal tibial osteolysis were noted on radiographs. The membranes around these four implants contained concentrations of macrophages that had phagocytized polyethylene and metal debris (Fig. 5). Biochemical results from specimens with radiographic evidence of osteolysis were also regrouped and compared with those specimens without evidence of osteolysis. The specimens from patients with evidence of osteolysis had released significantly higher ($p < 0.05$) amounts of collagenase, IL-

TABLE 2. Levels of Biochemical Factors in Experimental and Control Groups

	Cemented Mean ± SEM (range)	n	Cementless Mean ± SEM (range)	n	Control Mean ± SEM (range)	n
Collagenase* (units/g)	5.79 ± 4.47 (1.2–13.7)	23	5.93 ± 5.17 (1.8–14.4)	11	2.3 ± 1.2 (0–7.3)	10
PGE_2* (ng/mg)	5.52 ± 3.75 (0–13.9)	23	4.94 ± 3.83 (1.4–14.3)	11	1.93 ± 1.01 (0–3.01)	10
IL-1* (u/100mg)	0.39 ± 0.82 (0–0.66)	11	0.52 ± 0.57 (0–3.9)	6	undetectable	10
IL-6* (ng/g)	102.6 ± 103.1 (0–311.7)	23	123.1 ± 108.4 (0–303.5)	11	38.3 ± 35.7	10
TNF-α* (ng/g)	2.03 ± 3.29 (0–9.4)	19	1.63 ± 1.48 (0–5.9)	8	0.07 ± 0.45	10

n represents number of specimens in which activity was detected.
* Significantly higher than control values ($p < 0.05$).

FIG. 5. Polarized microscopy of a specimen taken from Zones 1 and 2 demonstrates birefringent debris within multinucleated foreign-body giant cells and mononuclear histocytes. (Stain, hematoxylin and eosin; original magnification, ×100.)

1, IL-6, and TNF-α into the culture media than did the membranes from prostheses without focal osteolysis (Table 3). There were no statistically significant differences between the levels of PGE_2 in groups with and without osteolysis, however.

IMMUNOHISTOCHEMICAL FINDINGS

In the 34 membranes analyzed, numerous macrophages were stained with antimacrophage antibody and were located beneath the synovial cell lining or fibrin layer, which was adjacent to the implant surface (Fig. 6). In sections of all membranes from both experimental groups that were stained sequentially with anti-CD3 and anti-CD22 monoclonal antibodies, very few T lymphocytes and B lymphocytes, respectively, were evident. Many macrophages (Fig. 7), relatively large numbers of fibroblasts, and some endothelial cells were stained with anti-IL-1, IL-6, and TNF-α antibodies. There were no significant differences between the numbers of anti-IL-6–positive cells in cemented and cementless membranes. In contrast, the populations of macrophages, fibroblasts, and endothelial cells that stained with anti-IL-1, IL-6, and TNF-α antibodies were larger in specimens from patients with bone lysis than in those without this problem.

TABLE 3. Comparison of Chemical Mediators in Condition Media From the Membrane With and Without Osteolysis in Failed Cemented and Cementless Knee Prostheses

	Focal Osteolysis			
	Positive		*Negative*	
	Cemented (n = 21)	*Cementless (n = 9)*	*Cemented (n = 25)*	*Cementless (n = 13)*
Collagenase (unit/g)	6.82 ± 5.11*	7.00 ± 4.46*	4.92 ± 5.02	5.18 ± 4.62
PGE2 (ng/g)	5.86 ± 3.28	5.21 ± 3.22	5.22 ± 3.05	4.76 ± 3.73
IL-1 (U/g)	6.01 ± 3.85*	6.70 ± 2.57*	2.11 ± 6.84	3.70 ± 5.21
IL-6 (ng/g)	125.5 ± 86.3*	152.5 ± 92.6*	83.3 ± 96.1	102.7 ± 98.5
TNF-α (ng/g)	3.03 ± 4.54*	2.91 ± 4.21*	1.19 ± 2.02	0.75 ± 1.26

* $p < 0.05$; values are the mean ± SEM.

FIG. 6. Antimacrophage antibody staining of the specimen shown in Figure 2 demonstrates numerous macrophages beneath a fibrin layer, which is adjacent to the implant surface. (The implant surface is the upper part of the specimen.) (Original magnification, ×200.)

DISCUSSION

Late loosening of total joint prostheses has been considered primarily a biomechanical process. It has become increasingly apparent, however, that periprosthetic tissues and their production of a number of biochemical substances play an important role in aseptic loosening. These interface membranes contribute to the bone resorption and osteolysis associated with aseptic loosening. This process has been previously described in total hip arthroplasty and can occur with cemented or uncemented prostheses and with stable and unstable implants.[7,11,13,15,17,18–20,23,28]

Recently, Nolan and Bucknill[21] and Peters *et al.*[22] investigated osteolysis associated with total knee prostheses. Their histologic findings were similar to those previously found with osteolysis surrounding cementless hip prostheses. They noted submicronic particulate polyethylene or metal debris within macrophages and large pieces of polyethylene debris or particulate PMMA within foreign-body giant cells. The authors also observed large intracytoplasmic asteroid bodies within foreign-body giant cells, however. These giant cells form from a syncytium of macrophages in response to particulate debris too large to be phagocytized by a single cell. As-

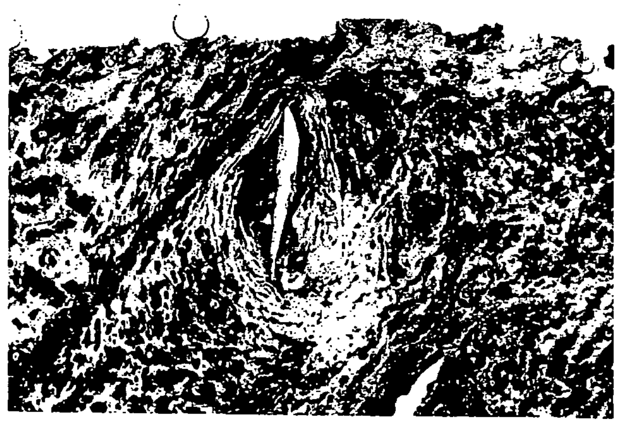

FIG. 7. Immunohistochemical staining of the membrane obtained from a patient with focal osteolysis using anti–TNF-α–positive antibody. Numerous anti–TNF-α–positive macrophages are visible. A large fragment of polyethylene is shown in the center of the field. (Original magnification, ×200.)

teroid bodies are composed of microtubules and intermediate fibers, two constituents of the normal cellular cytoskeleton.[1,2] Because asteroid bodies were seen in giant cells containing large particles of polyethylene debris, it is speculated that these particles create a microenvironment in which the giant cell cytoplasm undergoes various transformations from solution to a gelatinous form. These sol-gel transformations result in a condensation of the giant cell's cytoskeleton, which results in the formation of the stellate-shaped asteroid bodies. The presence of asteroid bodies may represent a burned out or "aged" population of giant cells.

Interestingly, large fragments of polyethylene, foreign-body giant cells, and fat were more common in the membranes obtained from failed total knee implants than in those from failed total hip implants, regardless of whether they were cemented or cementless. This may be due to the high contact stresses in knee implants and also may reflect the different motion of these implants. Knee implants have a very complicated motion, consisting of rolling, gliding, and sliding. In contrast, hip implants predominantly glide, producing small powderlike polyethylene and metal debris, which can stimulate macrophages, thus inducing a more rapid and extensive osteolysis than occurs in the knee. A different histologic and biochemical response in the interface membranes from failed cemented and cementless knee prostheses may explain the lower level of progressive osteolysis seen with these implants.

It was impossible to estimate how many particles each macrophage had phagocytized because of their irregularity, and because some macrophages were spread on the surface of the large particles. In addition, recent reports have indicated that many of these particles were too small to be resolved with light microscopy; therefore, microscopic analysis may not provide an accurate estimate of the amount of wear debris in the membranes.

Kim *et al.*[15] found substantial levels of collagenase, PGE_2, and IL-1 in the membranes from failed cementless femoral components in total hip arthroplasty—levels not significantly different from those seen in the membranes from failed cemented femoral components.[15] Likewise, no significant differences were found between cemented and cementless knee membranes in the levels of collagenase, PGE_2, IL-1, IL-6, and TNF-α. Nonetheless, in the current study, collagenase, IL-1, IL-6, and TNF-α levels were significantly higher in specimens from patients with focal osteolysis. The medial aspect of the tibial metaphysis was the most common site for osteolytic bone resorption, consistent with findings by Peters *et al.*[22] Although biochemical agents such as collagenase, PGE_2, IL-1, IL-6, and TNF-α are associated with bone resorption,[3,5,7,8,12,16] cytokines such as bone morphogenic protein and transforming growth factor-β should have been measured. Their activity may appear to be inhibited, because these cytokines are believed to stimulate osteoblasts.

It is well known that several biochemical mediators, including various cytokines, contribute to bone resorption.[9,10] It is unclear why membranes from patients with linear or lytic bone loss and those without bone loss did not contain significantly different levels of PGE_2, given that bone loss was associated with higher levels of all other biochemical factors measured. Interleukin-1 and TNF-α not only induce the production of IL-6 by macrophages, fibroblasts, and endothelial cells, but they also induce the proliferation of fibroblasts and endothelial cells.[24] Furthermore, IL-1 and TNF-α stimulate osteoclastic bone resorption. It was recently reported that IL-6 appears to induce osteoclastic bone resorption directly and to inhibit the proliferation of fibroblasts.[12,16,24] The biochemical and immunohistochemical results reported here suggest that macrophages, fibroblasts, and endothelial cells in the membranes react with metal or polyethylene debris and produce IL-6, which directly stimulates osteoclasts in patients with linear or lytic bone loss. Because IL-1 and TNF-α both stimulate os-

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teoclastic bone resorption[8,24] and induce IL-6,[9,10,12] IL-1 and TNF-α also may contribute directly as well as indirectly, through the induction of IL-6 production by macrophages, fibroblasts, and endothelial cells. It is unlikely that B lymphocytes and T lymphocytes contribute significantly to IL-6 production, because these two cell types were rarely observed in the current study. These observations suggest that significant levels of IL-6 activity were produced through a nonspecific chronic inflammatory reaction rather than through a lymphocyte-mediated immune response.

The results of this study demonstrate the ability of cementless and cemented knee membranes to produce similar amounts of the chemical agents associated with bone resorption. These factors may play a major role in the process of aseptic loosening of both cemented or cementless total knee prostheses. After the implantation of a prosthesis, various biochemical factors influence the surrounding cells. Abrasion of the articulation between the femoral component and the polyethylene articulating tibial and patellar surface as well as the micromotion of the prosthetic stem may result in the continual release of metal, particulate PMMA, and polyethylene debris. Subsequently, activated interface membranes can be produced by the foreign-body reaction initiated by this debris. Activation of various inflammatory cells such as macrophages and fibroblasts within the membrane can induce the production of a number of cytokines that are associated with bone resorption. The phagocytization of particulate polyethylene and metal debris by macrophages can induce the production of IL-1, IL-6, and TNF-α, which are considered important factors in bone resorption. This mechanism of aseptic loosening appears to play a role in failed knee as well as hip prostheses.

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